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THE SALK INSTITUTE

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Letter 2

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Dear Aaron,

Further to my earlier letter of April 27 I have been thinking more about nucleosome structure. I went back and looked at the Internal Memo (The Structure of the Solenoid) I sent you some months ago. The basic idea there was that there were about two turns of DNA per 200 bp and that the dyads of the nuclesomes were perpendicular to the axis of the solenoid, not as in Michael Levitt's model. Thus the general picture was not unlike Abe Worcel's except that I didn't arrive at his neat idea that different repeats alter the pitch and diameter of the solenoid. The basic question posed then (apart from the question of the number of DNA turns per solenoid, which, alas, is still with us) was whether the dyad point (the point when the nucleosome dyad goes through the DNA) was on the outside or the inside of the solenoid. I stated both alternatives and faintly favoured the inside. Worcel, in his blessed innocence, suggests the outside. The reasons (which he gives, and I also stated) are that the dyad in the linker is then also on the outside, and thus more accessible to enzymatic attack, as is the dyad point itself, for Zachau's enzymatic attack. Unfortunately neither of these arguments is conclusive, as explained in the memo.

Now it seems to me that your results on the density of the crystals, together with the e/m pictures, definitely favours the model with the dyad point on the outside of the solenoid. This gives a much more compact structure for 140 bp of DNA and could easily lead to views in which the two adjacent turns (strictly parts of turns) appeared approximately parallel. This is especially so if the DNA near the two ends of the 140 bp length is flattened a little. If the ends were flattened a lot it could make a "cylindrical" model; if flattened only moderately it would lead to a somewhat wedge-shaped cylinder of

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exactly the sort you propose. One also has to remember that the clipped core particle may well be modified a little at just these places. A moderately flattened model leads to something very close to 1 1/2 turns of a regular helix, with the pitch determined by the rather close packing on the inside of the solenoid. I must say I prefer this to your model of two rings joined by a bent connecting link, which seems forced to me.

In passing, did Len ever do a quantitative experiment, using his end labelling technique, to determine probabilities of cutting by DNase I on a clipped particle? You recall that from the sharpness or diffuseness of the lines on one of his gels we could tell that his results were roughly the same as on the unclipped particle, but did he ever do it with a label, to get exact probabilities? It would be of some interest to know if the clipping makes any difference.

In spite of the above I find it difficult to do more than guess how the DNA runs by these approaches.

On another point, I think you are too simplistic in your approach to the arrangement of the histones. Your diagrams look like Roger-diagrams to me. Experience has shown me that you must make histone packing models in 3D -- it's even difficult to visualize them in 2D using cylindrical projections, though these do help. Consequently, I think you are making too much of the e/m semi-some pictures. Note first that Chambon can only get them if he incubates nuclei and not, so far, by incubating the minichromose. This hints that something in the nucleus is needed to produce semi-somes. Unfortunately, the xerox copies I have of his pictures do not make it easy to examine them carefully but I feel sure that some histone arrangements within a platysome would split nicely to give the smaller size, especially if the semi-somes collapse a little on themselves. I would suggest that, just for fun, you try to make a 3D model of the clipped core particle, with rubber tube for the DNA and plasticene for the histones, TO SCALE. You might find it quite revealing.

I have myself made a tiny model of the DNA part (made from a paperclip) which has about 1 1/2 turns of what is approximately a regular helix (of pitch, say, 27 Å). What is interesting is the way two such models stack above one another -- they get much closer than you might think. This is because the DNA on the "top" of one does not make contact with the DNA "bottom" of the other, because, there being 1 1/2 turns, they are on opposite sides of the helix axis. Do try this. I have imagined them related by a translation parallel to the helix axis but the histones could easily impose a small angle, so that three in succession would give the slightly curved pack you have drawn. The point is that while the overall "height" in projection is at least 63 Å ((27 x 1 1/2) + 22) the

packing distance is appreciably smaller -- just 27 x 2 = 54 Å, in fact, not allowing for histone and making all DNA distance 27 Å. If one allowed a 22 Å approach between DNA in different nucleosomes the packing distance would be 27 + 22 = 49 Å. I am not suggesting that the DNA on the nucleosome is just over 1 1/2 turns of an exactly regular helix of pitch 27 Å but that a model approximately this, perhaps even with slightly "flared" ends to its DNA can pack with a packing distance of about 340/6 = 57 Å. In any case, I recommend the exercise to you. It seems to me to fit very well with all your suggestions. The point, I think, is that you do not need to have 2 complete turns (or two complete rings). About 1 1/2 turns are quite enough. This makes the packing potentially a lot more compact. There is one point you seem to be worrying about unnecessarily. This is the crosslinking result showing that adjacent (core) nucleosomes are in contact. This is easily explained if the contact is on the inner surface of the solenoid, where the distance between adjacent nucleosomes is necessarily small. Incidentally the apparent fact that H1 does not crosslink easily to the other histones (though very readily to other H1 molecules) does suggest that, at some point, there is a big space between adjacent core particles. Probably on the outside of the solenoid adjacent core particles are so far apart that H1 can sit there without touching the core particles on either side.

All the above is based on the assumption that solenoids are very regular though it seems likely to me that they really constitute a family of structures and thus can also form somewhat irregular structures. If the contact between adjacent turns of the solenoid is partly between one core particles and the ones above and below and partly between one H1 molecule and those H1's above and below, and if these contacts are somewhat flexible, then one easily obtains a family of solenoids. Whether one can obtain regular curved solenoids and whether this explains why H1 is less than equimolar with the other histones, I don't know. I can see how it might happen, but feel it's too early to worry about it much. Roger's result shows that the internucleosome spacing does really vary but what the variation is as one goes from nucleosome to nucleosome along the DNA remains to be seen. It could be random (unlikely). It could be that adjacent spacer sizes are correlated -- I think Garrod claims this. Or it could vary systematically as one might expect for a curved solenoid.

Garrod

Incidentally, do you use Roger's method of trimming (an exonuclease, followed by S1, rather than using only micrococcal DNase) to prepare core particles for crystalization, end labelling, etc. It might give a more uniform product, with less care needed to produce it, than the old method.

Enough for now,

Yours ever,

Francis

F. H. C. Crick
Ferkauf Foundation Visiting Professor

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